IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	Scott Walsh et al.		
Serial No.:	10/733,046	Group No.:	1645
Filed:	12/10/03	Examiner:	V.L. Ford
Entitled:	**Topical Anti-Infective Formulations**		

DECLARATION OF JAMES J. MOND, M.D., PH.D. UNDER 37 C.F.R. 1.132

EFS WEB FILED

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Examiner Ford:

1. I, James J. Mond, am one of the co-inventors of record of the subject matter embodied in the above-identified patent application. I am presently employed as Executive Vice President & Chief Scientific Officer at Biosynexus, Inc., the Assignee of the above-identified United States Patent Application.

2. I hereby provide the following comments regarding the pending application for patent:

It is alleged that Blackburn et al. Patent No. 5,762,948 teaches each and every element of the present invention, but this is not true. Blackburn mentions only once in passing that "other bacteriocin peptides like lysostaphin may also be suitably employed". What is suitably? There is no explanation or example in the patent as to how lysostaphin could be "suitably" employed. "Suitably" does not tell us anything about possible concentrations or ratios of lysostaphin to be used with a lantibiotic (e.g., nisin) as are described in the present patent application. Blackburn et al. also does not provide any empirical or experimental evidence for the topical use of lysostaphin alone or with a

lantibiotic (e.g., nisin). It is simply not possible to predict how two drug substances will interact beneficially or detrimentally without actually testing this experimentally. The Blackburn patent fails to provide any examples of compositions comprising a lantibiotic and lysostaphin, and also fails to provide any examples or experimental data using this type of composition in a method as provided in the present invention.

There is no way to know how two complex compounds like lysostaphin and nisin would interact when combined and whether they would be functional to treat a wound or skin infection as is claimed in the present application without experimental evidence. For example, it is not uncommon for two substances, that are beneficial when used independently, to either neutralize each other when combined, or even to have a detrimental effect rather than a beneficial one. The well known phenomenon of antibiotic antagonism describes the situation when the activity of two antibiotics is less than the activity of each used separately. It occurs primarily when a bacteriostatic antibiotic is used in combination with a bactericidal antibiotic. The bactericidal antibiotic requires a growing bacteria for it to exert its action and the bacteriostatic suppresses the growth of the bacteria without killing it. Thus, the numerous warnings on prescription drugs to not combine X with Y. This essential information is certainly not provided or even addressed by Blackburn.

Furthermore, Blackburn only discusses the use of nisin in wipes and not the various formulations of lysostaphin and lantibiotic/nisin that were actually generated and empirically tested in the present application and for which we provide experimental data that lysostaphin and nisin combined in the same formulation have efficacy for treating infections on the skin. In addition, the examiner is incorrect when they say that the Blackburn patent anticipates our claimed composition comprising 0.1-10 wt% lantibiotics (e.g., nisin) when Blackburn describes using 25ug/ml nisin. The weight percent (wt%) of the concentration of nisin used by Blackburn (25ug/ml) is actually <u>0.025 wt%</u> (10g/L=1 wt% or 10mg/mL=1 wt%, 10ug/uL=1 wt% and 25ug/mL=0.025 wt%) and this does not fall within our claimed weight percent. Based on this information, Blackburn et al. (No. 5,762,948) does not describe the invention of our application.

Claims are rejected based on the patent of Daley et al (No. 5,342,612) but Daley never discusses combining nisin and lysostaphin in one formulation, only that nisin

(again mentioned only in passing in column 4) is another example of a bactericidal agent that can be used to treat bovine mastitis as they describe. Daley does not provide any examples or experimental data of a method of decolonizing bacteria using a topical formulation with lysostaphin and a lantibiotic.

Daley does not show any examples or give any information on how lysostaphin could be combined with nisin and does not mention any other lantibiotics other than nisin. Furthermore, Daley incorrectly identifies lysostaphin as having bacteriostatic activity when it is well established in the art that lysostaphin is a rapidly bactericidal protein with the ability to enzymatically cleave the pentaglycine cross bridges of staphylococci leading to rapid lysis of bacteria. Daley incorrectly identifies the activity of lysostaphin in MIC assays as bacteriostatic, when that is not in fact how lysostaphin inhibits growth. Lysostaphin actually rapidly kills the initial inoculum in MIC assays. At the time of the invention, anyone familiar with the mechanism of lysostaphin acitivity would not have anticipated that it could work with nisin. The reason is that the lytic activity of lysostaphin on the bacterial membrane is so rapid on all of the bacteria that one might not expect any bacteria to be viable and available for killing by the slower action of the pore former nisin. Since Daley noted that lysostaphin is bacteriostatic, the assumption regarding the ability of lysostaphin to act in concert with other anti-infectives would be erroneous.

The Daley patent has examples that look at the potentiation of lysostaphin activity in vitro in response to various surfactants added to their specific formulation and is not relevant to our patent application. Furthermore, the in vivo examples provided by Daley are very different than what is being claimed in our patent application. In addition to lacking a lantibiotic, Daley actually infuses surfactant potentiated lysostaphin in various formulations <u>into</u> bovine mammary glands. This is very different than methods of using topical application of lysostaphin and one or more lantibiotics onto infected skin or wounds as claimed in our application. The interaction of lysostaphin in the milieu of a lactating mammary gland with the infecting bacteria in that gland as described in the Daley patent stands in stark contrast to the interaction of lysostaphin and a lantibiotic topically applied to infected skin or wounds as described in our patent application. For example, the skin is a very dry high salt environment and wounds contain many serum

and immune factors that are not expected to be found in lactating mammary glands of cows which are warm moist mucosal surfaces. There is no way to predict from Daley's patent concerning treatment by infusion into bovine mammary glands that methods of using lysostaphin together with a lantibiotic to topically treat infected wounds or skin would be successful.

Also, contrary to the allegation by the examiner, one of the aqueous surfactant vehicles described in Daley actually inhibited lysostaphin activity in bovine mammary glands (see Table 5 in column 13, lysostaphin in peanut oil, cures 0% vs. lysostaphin in saline cures 19%), further reducing the likelihood that the success of lysostaphin would be expected by one who looked to the Daley patent for guidance. Moreover, there are many examples of various antimicrobials in the literature which can successfully treat an infection in one body location or organ that are ineffective in another location or organ for various reasons. Two examples of this are mupirocin which is only effective topically but has no effect when used systemically (Suzanne F. Bradley. "Mupirocin". 2005. *In:* Antimicrobials Therapy and Vaccines Volume II: Antimicrobial Agents. VL Yu, G Edwards, PS Mckinnon, CA Peloquin and G Morse (*eds.*) ESun Technologies, Port City, MD.) and dalbavancin which is effective for treatment of bacteremia and right-sided endocarditis but is not effective for treating left-sided endocarditis or pneumonia (See, e.g., www.cubicin.com).

Thus, other than using lysostaphin to treat a very specific infection (bovine mastitis) by infusing surfactant potentiated lysostaphin formulations directly into the mammary glands of cows, Daley does nothing to support or describe the teachings of our patent application which uses lysostaphin and one or more lantibiotics to topically treat infections of skin and wounds.

The patent of Blackburn et al (4,980,163) describes bacteriocin compositions of lysostaphin and nisin and some other factors that may have a greater sum of activity than the parts alone when tested in milk at 37 °F. Milk is very different than infected skin or wounds. Milk contains certain proteins and other components that are very different than those found on infected skin or wounds as described in our patent application. Describing that lysostaphin plus nisin has additive activity against *S. aureus* in milk has no predictive value as to whether this combination would have such enhanced activity in

the complex environment of infected skin or wounds. Again, there are many examples in the literature of antimicrobials that appeared to have very good activity in vitro, only to have them fail in vivo when tested in animal models or on humans. Examples of this include: a) Anti-staphylococcal antibodies manufactured by two biotechnology companies which showed strong anti-staphylococcal activity in vitro but which possessed no efficacy when used in phase three clinical trials; and b)peptide antibiotics which were under development by Micrologix Inc. for nasal decolonization of *S. aureus* which demonstrated good in vitro efficacy against bacteria but which failed in clinical trials for nasal decolonization. Thus the Blackburn patent which only demonstrates the in vitro activity of lysostaphin and nisin in milk does nothing to describe how a combination of lysostaphin and a lantibiotic would work on the skin or in wounds.

In the second example of Blackburn, EDTA and surfactants are added to the formulation, but this would actually lead one away from our invention. Lysostaphin is a zinc containing enzyme and long term formulation of lysostaphin with EDTA could very well serve to inactivate the enzyme. In our patent application, it is only suggested to use EDTA with nisin alone. Thus neither Daley (which infuses lysostaphin combined with certain aqueous surfactants directly into the mammary gland of cows) nor Blackburn (which only describes that lysostaphin combined with nisin has activity in milk) supports or guides one to carry out the methods of using lysostaphin and one or more lantibiotics to treat infections of the skin or wounds of our application.

The examiner makes new rejections that are based on Patent No. 5,762,948 to Blackburn et al. As described above, the Examiner's characterization of Blackburn et al. was wrong. Blackburn does not provide any support or experimental data using a composition that has both lysostaphin and a lantibiotic. Also, the amount of lantibiotic (nisin) used by Blackburn (25ug/ml) is actually 0.025% wt% and does not fall into the 0.1 to 10.0 wt% of lantibiotic that is claimed in the present application. Furthermore, Blackburn only provides examples for using wipes formulated with nisin and does not provide any examples or experimental evidence using liquid formulations (separate from a wipe) as alleged by the examiner. Any liquids discussed by Blackburn are only used to formulate wipes by wetting them and are never demonstrated to have efficacy by themselves.

The examiner cites the patent of Gasson (No. 6,448,034) as teaching nisin variants that have improved properties compared with natural nisin for prevention of food spoilage. However, this patent is not at all relevant to the present patent application and there would be no reason why someone who attempted to do the work that we did that lead to the present patent application would ever use Gasson as a resource. Gasson does not even mention lysostaphin and does not provide any examples of treatment of any infections (e.g., with nisin).

The examiner also cites a patent by Krieger (No. 6,503,881). However, like Gasson, this patent does not even mention lysostaphin, or nisin, anywhere in the patent. Contrary to the Examiner's argument, one would not look to Krieger (or Gasson) to try to find guidance for a method of decolonizing bacterial populations using a topical that has both lysostaphin and a lantibiotic (e.g., nisin). There would be no reason for someone to consult or reference this patent as guidance for attempting to carry out the work that lead up to our invention. Nisin and the other lantibiotic peptides are not cationic or indolicidin peptides. While Krieger may teach that bacitracin suppresses colonization, the present application provides that lysostaphin and/or nisin treat infection. These are two very different things. It is not necessarily the case that a reagent that prevents bacterial colonization would also be suitable for treating or decolonizing bacteria.

3. The undersigned declares further that all statements made herein of his knowledge are true and that all statements made on information and belief are believed to be true; and further that those statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under § 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing therefrom.

Date: Oct 9, 2007

By: _____

James J. Mond, M.D., Ph.D.